Nitric oxide and its modulators in chronic constriction injury-induced neuropathic pain in rats

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Abstract

This study was conducted to examine the role of nitric oxide (NO) in peripheral neuropathy induced by chronic constriction injury of sciatic nerve of rats by using NO precursor, NO donors and nitric oxide synthase (NOS) inhibitors. Chronic constriction injury of sciatic nerve of rats resulted in peripheral neuropathy as confirmed by nociceptive behavioural tests using mechanical, thermal and cold allodynia. NO precursor, L-arginine and NO donors sodium nitroprusside, S-nitroso-N-acetylpenicillamine potentiated the hyperalgesia and allodynia significantly suggesting proalgesic effect in neuropathic rats. Intracerebroventricular (i.c.v.) administration of rats with NOS inhibitors such as L-NG-nitroarginine methyl ester, N-iminoethyl lysine and 7-nitroindazole did not show any effect but i.p. administration of NOS inhibitors aminoguanidine, L-NG-nitroarginine methyl ester and 7-nitroindazole caused alleviation of pain. The study confirms the involvement of endogenously synthesized and exogenously administered NO in chronic constriction injury-induced neuropathy in rats. Significant increase in the levels of nitrate and nitrite in ligated sciatic nerve suggest that local up regulation of NO in the production and maintenance of neuropathic pain. In conclusion, initial attempt to manipulate L-arginine: NO pathway is indicative of therapeutic potential of these interventions in the management of neuropathic pain.

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1. Introduction

Neuropathic pain is initiated by a primary lesion or dysfunction of the nervous system, which may be peripheral (peripheral nerve, plexus, nerve root) or central. The painful area within the territory of the injured nerve shows allodynia (hypersensitivity to normally non-painful stimuli), hyperalgesia and hyperpathia (Gordh, 1998), which are hallmark signs of neuropathic pain. The anatomical sites of lesions causing neuropathic pain are multiple. The most common locations are the peripheral nerves, the plexus, dorsal nerve roots, the spinal cord and brain.

The mechanism of neuropathic pain is still unclear in spite of extensive investigations. Several experimental models of peripheral mononeuropathy in rats were developed wherein chronic constriction injury (Bennett and Xie, 1988) or partial lesion (Seltzer et al., 1990) of sciatic nerve or its root (Kim and Chung, 1992) was done. The allodynia and hyperalgesia that develop, thereafter, have led to considerable advances in understanding neuropathic pain resulting from nerve injury. Further, changes were observed in pain behaviour with a peak occurring at 2–4 weeks post injury (Basile et al., 1993). Different pathological phenomenon might be operating with nerve injury. Accordingly, the present study was carried out to examine the role of nitric oxide (NO) by using NO precursor, L-arginine; NO donors, sodium nitroprusside and S-nitroso-N-acetyl penicillamine and NO synthase (NOS) inhibitors such as, L-NG-nitroarginine methyl ester, N-iminoethyl lysine, 7-nitroindazole and aminoguanidine in modulating the peripheral neuropathy and behavioural pain responses in rats with chronic constriction injury.

Neuropathic pain is much more difficult to treat than nociceptive pain. In the individual patient, neuropathic pain is usually chronic, spontaneous and/or stimuli evoked and sometimes impossible to treat (Gordh, 1998). Despite intensive research on the neurobiological mechanism of chronic pain, this
therapeutic area remains one of the least satisfactorily covered by current drugs. Effective therapy for this pain is lacking, and the underlying mechanisms are poorly understood (Tsuda et al., 2003).

Neuropathic pain is probably not based on a single pathophysiological process (Malmberg and Basbaum, 1998). It is often refractory to treatment with conventional analgesics such as, opiates and nonsteroidal anti-inflammatory drugs (Tanelian and Brose, 1991; Rowbotham, 1994). The use of opioids in neuropathic pain is controversial because of their limited efficacy in this pain state as compared to other pain states.

N-methyl-D-aspartic acid (NMDA) receptor antagonists, adenosine analogues, neuronal specific Ca\(^{2+}\) channels blockers and NO modulators are among the pharmacological tools that are being studied to find out new treatment strategies (Gordh, 1998). Activation of NMDA receptor is associated with increased intracellular Ca\(^{2+}\) concentration and activation of Ca\(^{2+}\) sensitive protein kinase C, resulting in the production of NO, which produces persistent enhancement of pain. It is now believed that the mechanism responsible for hyperalgesia in chronic pain may involve not only NO itself, but also the product of its reaction with superoxide radicals, the peroxynitrite (Tal, 1996). The present study is therefore, aimed at understanding the role of NO in chronic constriction injury-induced neuropathy in rats.

2. Materials and methods

2.1. Animals

Adult male albino rats (175–225 g) of Wistar strain obtained from Laboratory Animal Resource Section of Indian Veterinary Research Institute were used in the present study. The animals were housed in groups of 5–6 in colony cages for one week till chronic constriction injury of sciatic nerve was done. After surgery, the rats were kept in individual cages at room temperature of 25±2 °C during which the animals were repeatedly and gently handled to get them acclimatized to the laboratory environment and stress. A balanced rat feed obtained from Feed Technology Unit of the Institute and clean drinking water were provided ad libitum. The experimental procedures were approved by the Institute Animal Ethics Committee.

2.2. Induction of chronic constriction injury

Selected rats were kept off feed for 12 h prior to surgery. After induction of anesthesia with ketamine hydrochloride (100 mg/kg, i.m.), the hair around the mid-thigh were clipped and then shaved. Chronic constriction injury was induced as described by Bennett and Xie (1988). The common sciatic nerve of right hind limb was exposed at the level of the middle of the thigh by blunt dissection through biceps femoris. Proximal to the sciatic trifurcation, about 7 mm of the nerve was freed off the adhering tissue and four ligatures (4.0 silk) were tied loosely around it with about 1 mm spacing, with the length of the affected nerve being 4–5 mm long. The desired degree of constriction was such that it could retard, but not arrest circulation through the superficial epineural vasculature. The incision was closed in layers. In sham-operated rats, an identical surgery was performed except that the sciatic nerve was not ligated.

After suturing the skin, povidone iodine solution was applied externally by cotton swab and prophylactically, oxytetracycline (Terramycin, Pfizer, India) was injected i.m. at a dose of 50 mg/kg body weight for three consecutive days to prevent any infection. The operated animals were caged individually and feed and water were given ad libitum. They were allowed to recover for two weeks before drug administration/excision of nerve was undertaken.

2.3. Intracerebroventricular (i.c.v.) cannulation

The experimental rats were cannulated i.c.v. (Verster et al., 1971) under proper aseptic conditions. For this purpose, the rats were fasted for 12 h prior to surgical procedure. The animals were anesthetized with ketamine hydrochloride (100 mg/kg, i.m.).

An incision was made over the mid-line of skull of about 3 cm approximately, from anterior to the posterior direction and a small burr hole was drilled at the coordinates, 2 mm lateral and 1 mm caudal towards the right hand side of the bregma mark over the skull. Two stainless steel screws were fixed to the skull taking care that they do not pierce the bone, one at 1 mm behind and the other 1 mm in front of the bregma mark. A 47 number polyethylene guide cannula, filled with artificial cerebrospinal fluid was inserted through the small hole to a depth of 4 mm below the skull surface. The cannula was fixed to the skull surface with the help of stainless steel screws and dental cement. The hold up capacity of the cannula was 5 μl. After fixing, 5 μl of artificial cerebrospinal fluid was passed through the cannula into the right lateral ventricle to clear the passage. Finally, the top of the cannula was sealed by mild heat. The operated rats were allowed to recover for 7 days and divided into groups of six animals each for control and different pharmacological experiments.

After the termination of the experiment, all the rats were injected i.c.v. with 5 μl of 1% Evan’s blue solution in order to ascertain the correct position of the cannula in the right lateral ventricle of the brain.

2.4. I.C.V. administration of drugs

The drug solutions were prepared afresh just before i.c.v. administration, by dissolving them in sterile artificial cerebrospinal fluid, except 7-nitroindazole which was dissolved in arachis oil. The concentration of different drugs in artificial cerebrospinal fluid was adjusted in such a way that a constant volume of 5 μl of the drug solution was injected i.c.v. by cutting the sealed top of the cannula at the rate of 1 μl/min, using 10 μl syringe. Then, 5 μl of artificial cerebrospinal fluid was administered to push the full amount of drug into the ventricle.
and the cannula was resealed. Untreated control group of rats were administered i.c.v. with 5 μl of artificial cerebrospinal fluid/arachis oil.

The doses of NO modulators used in the present study are summarized in Table 1.

2.5. Recording of pain threshold

2.5.1. Mechanical stimulation

The pressure in g was recorded as pain threshold by Randall–Selitto assay method (1957) using Randall–Selitto analgesiometer (UGO Basile, Varese, Italy), immediately prior to the administration (0 h) of above mentioned drugs in chronic constriction injury induced rats, and at 1, 3, 5 and 7 h after drug administration (i.c.v. or i.p.). The cut off pressure was 150 g. The change in pain threshold in test group was compared with that of cerebrospinal fluid treated chronic constriction injury control groups at corresponding time. Results are expressed as mean pressure in grams±S.E.M.

2.5.2. Radiant heat

The latency to radiant heat in s was measured by radiant heat apparatus, (UGO, Basile, Varese, Italy), immediately prior to (0 h) and at 1, 3, 5 and 7 h after drug administration. The paw was placed on the heat radiator and the withdrawal of paw was measured in seconds. The change in paw withdrawal latency of test group was compared with that of cerebrospinal fluid treated chronic constriction injury control groups at the corresponding time. Results are expressed as mean time in seconds±S.E.M. The cut off time was 15 s.

2.5.3. Cold allodynia

Ice cool water (4±1 °C) was taken in a beaker. The right paw of chronic constriction injury-induced drug treated rats (test group) and untreated rats (control group) was submerged gently in the water and the withdrawal time (in seconds) was measured. The change in paw withdrawal latency of test group was compared with that of cerebrospinal fluid treated chronic constriction injury control groups at the corresponding time. Results are expressed as mean time in seconds±S.E.M. The cut off time was 20 s.

2.6. Estimation of nitrate/nitrite

Sciatic nerves from chronic constriction injury induced and sham operated rats were obtained on the 15th day of surgery. A segment of sciatic nerve approximately 1.5 cm in length, 5 mm proximal and 5 mm distal to the injured site was used for preparing the homogenate at a concentration of 1:100 for estimation nitrate and nitrite. Blood was collected for the separation of serum for estimation of nitrate and nitrite. Nitrate and nitrite in the sciatic nerve homogenate was estimated following the procedure described by Sastry et al. (2002). The principle of assay is the conversion of nitrate to nitrite by copper–cadmium alloy and then development of colour by Greiss reagent (sulfanilamide and N-1-naphthyl ethylene diamine) in acidic medium. By using standard curve, the amount of nitrate and nitrite present in the sample was calculated and the results are expressed in micromoles per gram of tissue or micromoles per milliliter of serum.

2.7. Statistical analysis

The results of NO modulators and nitrate and nitrite levels were analysed by analysis of variance (ANOVA) followed by Studentized range test. p<0.05 was considered significant.

3. Results

3.1. Behavioural observations

The rats with chronic constriction injury developed abnormal gait, posture, guarding and protective behaviour and licking of the hindpaw of the ipsilateral side of sciatic ligation after 1–2 days of operation. The rats could not put weight on the affected side and the hind limb of the affected side was drawn close to the body with distinctive guarding posture. The foot was markedly ventroflexed and the toes were held together tightly. The abnormal behaviour was observed even after 2 weeks of operation.

3.2. Effect of i.c.v.-administered NOS inhibitors (L-NG-nitroarginine methyl ester, N-iminoethyl lysine and 7-nitroindazole) on chronic constriction injury-induced neuropathic rats

There was no significant effect of L-NG-nitroarginine methyl ester, N-iminoethyl lysine and 7-nitroindazole at 50, 100 and 200 μg/rat doses, on pain threshold of neuropathic rats assessed by mechanical, radiant heat and cold stimulation (Figs. 1, 2 and 3).

3.3. Effect of i.p.-administered NOS inhibitors (aminoguanidine, L-NG-nitroarginine methyl ester and 7-nitroindazole) on pain threshold of neuropathic rats

3.3.1. Effect on mechanical stimulation

Aminoguanidine at 100 and 300 mg/kg i.p. increased the pain threshold on mechanical stimulation in neuropathic rats
significantly from 3 to 7 h of observation (Fig. 4A). However, 30 mg/kg dose did not alter the pain threshold. Similarly, L-N^G-nitroarginine methyl ester at 3 and 10 mg/kg did not alter the mechanical pain threshold, but at 30 mg/kg dose, it significantly increased the pain threshold from 1 to 5 h of observation (Fig. 4B).

7-nitroindazole at 3 mg/kg dose did not have any significant effect on the pain threshold, but at 10 and 30 mg/kg, it increased the pain threshold significantly at 3 to 5 h and from 1 to 7 h of observation, respectively (Fig. 4C).

### 3.3.2. Effect on radiant heat stimulation

The results of i.p. administration of aminoguanidine, L-N^G-nitroarginine methyl ester and 7-nitroindazole on radiant heat stimulation of hind limb of chronic constriction injury-induced neuropathic rats are summarized in Fig. 5. Aminoguanidine at 100 and 300 mg/kg increased the reaction time significantly from 1 to 5 h and 1 to 7 h, respectively to radiant heat stimulation (Fig. 5A). However, aminoguanidine at 30 mg/kg dose, did not show any effect on the pain threshold up to 7 h of observation.

L-N^G-nitroarginine methyl ester in doses of 3 and 10 mg/kg did not alter the pain threshold in neuropathic rats. However, L-N^G-nitroarginine methyl ester at 30 mg/kg dose, increased significantly the reaction time to radiant heat stimulation at 3 to 5 h of post drug administration (Fig. 5B).

7-nitroindazole at a dose rate of 3 mg/kg did not have any effect on the pain threshold, while at 10 and 30 mg/kg, it increased the reaction time significantly at 3 h (Fig. 5C).
3.3.3. Effect on cold-allodynia

Aminoguanidine at a dose rate of 30 mg/kg did not show any effect on the pain threshold to cold stimulation. However, at 100 and 300 mg/kg doses, there was significant increase in reaction time to cold stimuli (Fig. 6A) from 1 to 7 h of observation.

L-NG-nitroarginine methyl ester at 3 mg/kg dose increased the reaction time to cold stimuli significantly at 7 h of observation. Similarly, at 10 and 30 mg/kg, it increased the reaction time from 5 to 7 h and 1 to 7 h of observation, respectively (Fig. 6B).

7-nitroindazole at 3 mg/kg dose, did not significantly increase the reaction time. At a dose rate of 10 mg/kg, 7-nitroindazole increased the reaction time significantly from 5 to 7 h of observation (Fig. 6C). At 30 mg/kg dose rate, 7-nitroindazole reduced the pain from 1 to 7 h of observation.

3.4. Effect of i.p. administered NO precursor L-arginine on pain threshold in chronic constriction injury-induced neuropathic rats

3.4.1. Effect on mechanical stimulation

L-arginine, in doses of 0.3, 0.5 and 1.0 mg/kg increased hyperalgesia in neuropathic rats, the peak effect being at 1 h post drug administration and the hyperalgesia decreased gradually up to 7 h of observation (Fig. 7A).

3.4.2. Effect on radiant heat stimulation

L-arginine administered at dose rates of 0.3, 0.5 and 1.0 mg/kg i.p., caused significant increase in hyperalgesia. The peak effect was observed at 1 h post drug administration which decreased gradually up to 7 h of observation (Fig. 7B).
3.4.3. Effect on cold allodynia

L-arginine, at 0.5 and 1.0 mg/kg doses decreased the threshold to pain by cold stimulation. The peak hyperalgesia was observed at 1 h of observation which gradually decreased up to 7 h of observation (Fig. 7C).

3.5. Effect of NO donors SNP and SNAP on pain threshold in chronic constriction injury-induced neuropathic rats

3.5.1. Effect on mechanical stimulation

Sodium nitroprusside and S-nitroso-N-acetylpenicillamine at 0.3 mg/kg dose, did not alter the pain threshold to mechanical stimulation in neuropathic rats. However, sodium nitroprusside at 1.0 and 3.0 mg/kg dose reduced the pain threshold significantly from 3 to 7 h post drug administration (Fig. 8A) with the peak effect being at 5 h of post drug administration.

S-nitroso-N-acetylpenicillamine at 1.0 and 3.0 mg/kg also increased hyperalgesia up to 7 h of observation with a peak effect at 5 h (Fig. 8B).

3.5.2. Effect on radiant heat stimulation

Intraperitoneal administration of 0.3 mg/kg sodium nitroprusside was not effective in increasing pain withdrawal latencies to radiant heat stimulation. However, sodium nitroprusside at 1 mg/kg dose reduced the pain threshold significantly at 3 h of observation. Sodium nitroprusside at a threshold...
dose rate of 3.0 mg/kg, decreased pain withdrawal latencies significantly with a peak at 5 h of observation as evidenced from Fig. 9A.

S-nitroso-N-acetylpenicillamine at 0.3 mg/kg dose had no effect on pain withdrawal latencies to radiant heat stimulation. However, S-nitroso-N-acetylpenicillamine at doses of 1.0 and 3.0 mg/kg reduced the duration of paw withdrawal to radiant heat significantly with a peak effect at 3 h of observation (Fig. 9B).

3.5.3. Effect on cold allodynia

Intraperitoneal administration of sodium nitroprusside and S-nitroso-N-acetylpenicillamine at doses of 0.3, 1.0 and 3.0 mg/kg did not alter the pain behaviour in neuropathic rats.

3.6. Assessment of nitrate and nitrite in the nerves of chronic constriction injury-induced neuropathic rats

The amount of nitrate and nitrite in the serum was 49.00 ± 2.38 and 48.33 ± 2.03 μmol/ml in naïve and chronic constriction injury-induced neuropathic rats, respectively. There was no significant difference in the nitrate and nitrite in the sciatic nerve of naïve control rats (56.16 ± 2.26 μmol/g) and in the contralateral (sham operated) nerve of the chronic constriction injury-induced rats (60.00 ± 1.36 μmol/g). But, a significant rise in the nitrate and nitrite levels was noted in the ligated nerve (84.16 ± 2.66 μmol/g), as compared to sham operated nerve (60.00 ± 1.36 μmol/g) and naïve control nerve (56.16 ± 2.20 μmol/g).

4. Discussion

Several models of painful neuropathy have been developed in rats in recent years to study the mechanism of development and maintenance of allodynia and to assess the effect of various treatments (Kim and Chung, 1992; Kim et al., 1997). Among these neuropathic pain models, chronic constriction injury of sciatic nerve in rats has been widely used as it produces reliable and sustained tactile allodynia which resembles the conditions observed in patients with neuropathic pain (Bennett and Xie, 1988). In the present investigation, rats showed a significant reduction in thermal, mechanical and cold thresholds in hind
Neuropathic pain is not a single entity. It is heterogeneous in nature. The role of NO in neuropathic pain is uncertain, although there are several lines of investigation suggesting that spinal NO is pronociceptive (Terenghi et al., 1993; Yang et al., 1996). On the contrary, spinal NO is reported to be involved in the antinociception produced by morphine (Kolesnikov et al., 1997; Song et al., 1998). Since neuropathic pain is often unresponsive to conventional treatment (Amer and Meyerson, 1988; Ossipov et al., 1995), investigation into the role of NO in the pathophysiology of chronic nerve ligation (Cizkova et al., 2002) is warranted. As NOS activity is upregulated in chronic constriction injury-induced neuropathic rats, there is enhanced production of NO, which is metabolized to nitrate and nitrite. Estimation of nitrate and nitrite is thus, a convenient and simple way of expression of NO activity.

Peripheral nerve injury is also associated with local up regulation of iNOS in macrophages and Schwann cells with subsequent NO release. NO participates in the response of the peripheral nerve injury by generating increase in nerve blood flow within the injured nerve trunk (Levy et al., 1999). The expression of iNOS and local elaboration of NO may either promote or protect against neuronal damage (Sinz et al., 1999).

Fig. 9. Effect of intraperitoneally administered sodium nitroprusside (A) and L-nitroso-N-acetylpenicillamine (B) on pain produced by radiant heat stimulation of hind limb of chronic constriction injury-induced neuropathic rats. Reaction time was recorded 1h after i.p. administration of drugs. The vertical lines at the top of the bars represent the S.E.M. n=6. *p<0.05 compared with control (ANOVA).

As it is evident from the literature, there is increased nitrate and nitrite in naive and chronic constriction injury-induced neuropathic rats. We observed a significant rise in nitrate and nitrite levels in the sciatic nerves of chronic constriction injury-induced neuropathic rats, compared to naive and sham rats. But there was no difference in nitrate and nitrite levels in the serum of both naive and chronic constriction injury-induced neuropathic rats. This implies that the local increase of NO in nerve is important for the maintenance of pain following nerve injury. Nitrate and nitrite levels are significantly increased both in the cerebellum and brain stem of rats with neuropathic pain when compared with healthy rats (Onal et al., 2003). The study further suggests that NOS activity is up regulated in neuropathic pain in sciatic nerve.

In the present study, we estimated nitrate and nitrite in naive and chronic constriction injury-induced neuropathic rats. We observed a significant rise in nitrate and nitrite levels in the sciatic nerves of chronic constriction injury-induced neuropathic rats, compared to naive and sham rats. But there was no difference in nitrate and nitrite levels in the serum of both naive and chronic constriction injury-induced neuropathic rats. This implies that the local increase of NO in nerve is important for the maintenance of pain following nerve injury. Nitrate and nitrite levels are significantly increased both in the cerebellum and brain stem of rats with neuropathic pain when compared with healthy rats (Onal et al., 2003). The study further suggests that NOS activity is up regulated in neuropathic pain in sciatic nerve.

As it is evident from the literature, there is increased production of NO coupled with increased expression of NOS and associated hyperalgesia caused by nerve injury. Therefore, in the present investigation, we used L-N^G-nitroarginine methyl ester (L-NAME) to study the effect of NO precursor on neuropathic pain, (iii) to study the effect of NO donors on neuropathic pain and (iv) to estimate the NOS activity by indirect method (nitrite and nitrate content) of the affected nerve.

Different alterations of NOS immunoreactivity in the lumbar dorsal root ganglions and spinal cord of rat and monkey were described after peripheral axotomy (Zhang et al., 1993). In a model of peripheral neuropathy induced by ligation of the left L5 and L6 nerve roots, NOS activity was increased in the ipsilateral L5 and L6 dorsal root ganglions following neuropathic surgery. The changes in NOS activity in the dorsal root ganglion were observed 2 weeks after the nerve injury at the level of the injured spinal nerves, but not in adjacent dorsal root ganglions corresponding to uninjured spinal nerves. These observations provide evidence that local changes in NOS activity are relevant to the genesis and/or maintenance of altered pain behaviour (Choi et al., 1996). Increased production of NO in particular, may be involved in the development of neuropathic pain related behaviour in animals after peripheral nerve injury. Thus, peripheral nerve section induced an upregulation of NOS activity in ipsilateral dorsal root ganglion cells (Steel et al., 1994; Verge et al., 1994).

Peripheral nerve injury is also associated with local up regulation of iNOS in macrophages and Schwann cells with subsequent NO release. NO participates in the response of the peripheral nerve injury by generating increase in nerve blood flow within the injured nerve trunk (Levy et al., 1999). The expression of iNOS and local elaboration of NO may either promote or protect against neuronal damage (Sinz et al., 1999).

It has been shown that ligation of the spinal cord of rats resulted in an up regulation of NOS which is most pronounced in the ipsilateral gray matter of the spinal cord compared to the contralateral side, suggesting a putative role of NO in the prevention of neuronal damage (Sinz et al., 1999). As iNOS is increased in chronic constriction injury-induced rats, there is enhanced production of NO, which is metabolized to nitrate and nitrite. Estimation of nitrate and nitrite is thus, a convenient and simple way of expression of NOS activity.

In the present study, we estimated nitrate and nitrite in naive and chronic constriction injury-induced neuropathic rats. We observed a significant rise in nitrate and nitrite levels in the sciatic nerves of chronic constriction injury-induced neuropathic rats, compared to naive and sham rats. But there was no difference in nitrate and nitrite levels in the serum of both naive and chronic constriction injury-induced neuropathic rats. This implies that the local increase of NO in nerve is important for the maintenance of pain following nerve injury. Nitrate and nitrite levels are significantly increased both in the cerebellum and brain stem of rats with neuropathic pain when compared with healthy rats (Onal et al., 2003). The study further suggests that NOS activity is up regulated in neuropathic pain in sciatic nerve.
ester, N-iminoethyl lysine and 7-nitroindazole by i.c.v. route and aminoguanidine, L-N\(^{G}\)-nitroarginine methyl ester and 7-nitroindazole by i.p. route to assess the effect of NOS inhibitors on pain perception in chronic constriction injury-induced neuropathic rats. The i.c.v. administration of L-N\(^{G}\)-nitroarginine methyl ester, N-iminoethyl lysine and 7-nitroindazole did not affect the pain perception because NOS inhibitors were injected ipsilaterally in the ventricle to ligated sciatic nerve (right). The i.c.v. administration of L-N\(^{G}\)-nitroarginine methyl ester ipsilateral to the ligated paw inhibited NOS in the somatosensory cortex responsible for processing afferent; the residual of NOS activity appears to be sufficient for nociception (Salter et al., 1996). This is consistent with other studies showing that a number of distinct NO-dependent behavioral responses are only affected when NOS is inhibited by greater than 50\% (Kapas et al., 1994; Salter et al., 1995). However, antihyperalgesic effect of L-N\(^{G}\)-nitroarginine methyl ester was observed when it was administered contralateral to the ligated caudal sural cutaneous nerve and therefore, ipsilateral to cortical nociceptive processing from this nerve (Salter et al., 1996). However, L-N\(^{G}\)-nitroarginine methyl ester has been found to be antinociceptive in various pain models following i.c.v. administration (Przewlocka et al., 1994; Sarma, 2000). Besides, ipsilateral administration to ligated sciatic nerve, reason for failure of L-N\(^{G}\)-nitroarginine methyl ester, N-iminoethyl lysine and 7-nitroindazole on i.c.v. administration may be due to differences in the pathophysiological mechanism of nociception and neuropath. The possibility of antinociceptive effect of L-N\(^{G}\)-nitroarginine methyl ester and other NOS inhibitors administered i.c.v. in lateral ventricles, contralateral to ligated sciatic nerve could not be ruled out from present investigation.

Intraperitoneal administration of aminoguanidine, L-N\(^{G}\)-nitroarginine methyl ester and 7-nitroindazole alleviated the pain significantly in chronic constriction injury-induced neuropathic rats. Aminoguanidine is selective iNOS inhibitor and there is no report of its use in neuropathic pain in rodent model. In the present study, aminoguanidine reduced the hyperalgesia due to mechanical stimulation. Similarly, aminoguanidine was also able to delay the hyperalgesia in radiant heat and cold assay procedures. Peripheral nerve injury is associated with up-regulation of iNOS in macrophages and Schwann cells within and distal to injury site (Levy et al., 1999). The local increase of iNOS mRNA and expression paralleled with both temporal and spatial protein expression. Thus, iNOS-NO is the key mediator in neuropathic pain, and its inhibition by aminoguanidine is responsible for reduction of hyperalgesia in chronic constriction injury-induced rats.

L-N\(^{G}\)-nitroarginine methyl ester, non-selectively blocks all isoforms of NOS, whereas 7-nitroindazole is a selective nNOS inhibitor (Hao and Xu, 1996). Systemic administration of L-N\(^{G}\)-nitroarginine methyl ester and 7-nitroindazole alleviated chronic allodynia like symptoms in chronic constriction injury-induced neuropathic rats in this study. Similar observations have been recorded in thermal hyperalgesia in chronic constriction injury-rats when L-N\(^{G}\)-nitroarginine methyl ester was applied directly and continuously to the site of chronic constriction injury via osmotic pump (Thomas et al., 1996).

Related studies also confirm attenuating the effect of L-N\(^{G}\)-nitroarginine methyl ester in various models of neuropathic pain (Meller et al., 1992; Hao et al., 1994; Yamamoto and Shimoyama, 1995). Similarly, 7-nitroindazole was found to be antinociceptive in various models of neuropath (Allawi et al., 1994; Hao and Xu, 1996). These results suggest the involvement of NO in the development and maintenance of hyperalgesia in rat model of neuropathy. Our observations demonstrated that local production of NO is important for hyperalgesia in nerve injury than central production in neuropathic rats which is supported by reported literature.

L-arginine (2-amino-5-guanidinvaleric acid) is a basic, semi-essential amino acid (pH 5–6.5, Boger and Bode-Boger, 2001). NO is synthesized from substrate L-arginine by isoforms of NOS and produces hyperalgesia after activation of the NMDA receptor (Kitto et al., 1992). NOS is inhibited by L-arginine analogs that are substituted at the guanido nitrogen atom, like \(N^G\)-monomethyl-L-arginine or \(N^G\)-nitro-L-arginine. Inhibitory action of these molecules is overcome by excess L-arginine. When L-arginine was administered intrathecally, autotomy behaviour was observed which was attenuated by intrathecal treatment with L-N\(^{G}\)-nitroarginine methyl ester (Niedbala et al., 1995).

In the present investigation, administration of L-arginine intraperitoneally to neuropathic rats resulted in hyperalgesia (as measured by mechanical, radiant heat and cold stimulation). The peak hyperalgesic effect of L-arginine was observed at 1 h post drug administration. The data proves the hyperalgesic action of NO in neuropathic rats. Similar hyperalgesic effect of L-arginine was seen in different nociceptive models (Moore et al., 1993; Honore et al., 1995; Takano et al., 1998).

We used NO donors sodium nitroprusside and S-nitroso-N-acetylpenicillamine intraperitoneally at dose rates of 0.3, 1.0 and 3.0 mg/kg in neuropathic rats and measured the pain perception by mechanical, radiant heat and cold stimulation up to 7h post drug administration. From the data, it is evident that both the donors are hyperalgesic to neuropathic rats and a peak effect was shown at 3–5 h post drug administration. Hyperalgesia to mechanical and radiant heat was observed, but no significant effect on pain perception to cold stimulus was observed. Though, S-nitroso-N-acetylpenicillamine (0.2 \(\mu\)g) evoked hyperalgesia in the rat paw pressure and tail flick tests (Przewlocka et al., 1994), this is the first study on the hyperalgesic effect of S-nitroso-N-acetylpenicillamine in chronic pain. Our results are substantiated by another NO donor, nitroglycerine which on administration systemically induced a significant reduction in the latency of tail flick at 2 and 4h after its administration in formalin induced pain-related behaviour (Tassorelli et al., 2003).

In conclusion, the present study suggests that the increased production of NO locally is important in maintenance of pain in neuropathic rats which is alleviated by NOS inhibitors. The hyperalgesia is increased by NO precursor L-arginine and NO donors sodium nitroprusside and S-nitroso-N-acetylpenicillamine. Initial attempts to manipulate L-arginine: NO pathway is indicative of therapeutic potential of these interventions in the management of neuropathic pain.
References


